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(57) Abstract

A novel protein kinase, leucine-zipper protein kinase, 668 amino acids in length is provided by the present invention. This protein kinase is localized to the human brain. Nucleic acid sequences encoding the protein kinase are also provided.

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NOVEL PROTEIN-KINASE, NUCLEIC ACID SEQUENCES ENCODING THE SAME AND METHODS RELATED THERETO

Cross Reference to Related Applications

This is a continuation in part of U.S. Serial No. 08/205,018 filed March 1, 1994, the contents of which are incorporated by reference herein in their entirety.

Field of the Invention

This invention is directed to a novel protein-kinase, nucleic acid sequences encoding the same and methods related thereto.

Reference to Government Grants

The work present herein was supported in part by National Institute of Health grants NS08075, NS25044 and NS31102. The United States government may have certain rights in the invention.

Background of the Invention

Protein kinases regulate various cellular responses to changing environmental conditions. Protein kinases fall into two general classes: those protein kinases that transfer phosphate to serine or threonine and those proteins that transfer phosphate to tyrosine (Krebs and Beavo, *Annu. Rev. Biochem 48*: 923-959 (1979)). A few protein kinases, such as weel, now appear to be capable of phosphorylating both ser/threonine and tyrosine (Lindberg et al., *Trends Biochem Sci 17*: 114-119 (1992)). Phosphorylation is of particular significance in controlling

mitogenesis and cellular differentiation. Receptors for a number of polypeptide growth factors are transmembrane tyrosine kinases (Yarden and Ullrich, *Annu. Rev. Biochem 57*: 443-478 (1988)), which in turn stimulate serine/threonine kinases such as protein kinase C, MAP kinase and p74^{raf}(Hunter et al., *Nature 311*: 480-483 (1984); Morrison et al., *Cell 58*: 649-657 (1989); Rossomondo et al., *Proc. Natl. Acad. Sci. USA 86*: 6940-6943 (1989)).

Protein kinases, and especially the overexpression thereof, have been found to be linked to hyperproliferation of cells and metastasis. Many protein kinases were first identified as the products of oncogenes and still constitute the largest family of known oncogenes. Lindberg and Hunter, *Mol. and Cell. Biol.*, 10(11): 6316-6324 (1990).

Mutations of genes encoding members of the protein kinase family which are involved in the regulation of neuroblastic proliferation, differentiation and survival play a role in the etiology of human central nervous system tumors. Thus, it is highly desirable to gain a greater understanding of this class of proteins, as well as to use such greater understanding to limit or inhibit the effects that these proteins have on cellular hyperproliferation.

Summary of the Invention

There is provided by the present invention a cDNA sequence encoding a novel protein kinase, leucine-zipper protein kinase (zpk), and the protein encoded thereby.

There are provided by the present invention recombinant constructs encoding leucine-zipper protein kinase.

There are provided by the present invention novel methods of use and diagnosis for leucine-zipper protein kinase and cDNA coding for leucine-zipper protein kinase.

Brief Description of the Drawings

Figure 1. Nucleotide sequence (SEQ ID NO: 1) and putative amino acid sequence (SEQ ID NO: 2) of leucine-

zipper protein kinase. Amino acid numbering starts with the initiation codon.

Figure 2. Northern blots of expression of leucine zipper protein kinase in human tissue. Panel A represents Northern blots hybridized to α-[$_{32}$P] labeled leucine zipper protein kinase cDNA from human adult tissue (a) and from human fetal tissue (b). Panel B represents Northern blots hybridized to α-[$_{32}$P] labeled β-actin cDNA from human adult tissue (a) and from human fetal tissue (b).

Detailed Description of the Invention

A novel member of the protein serine/threonine kinase family, leucine-zipper protein kinase is provided by the present invention. As used herein, the term leucine-zipper protein kinase (zpk) refers to a protein having an amino acid sequence substantially homologous to at least a portion of the amino acid sequence set forth in SEQ ID NO: 2. In accordance with the present invention, the term "homologous" refers to a one to one correlation between the sequences of two polypeptides or oligonucleotides. Of course, 100% homology is not required in all cases. In some instances polypeptides of the present invention may be substantially homologous to the amino acid sequence set forth in SEQ ID NO: 2. Substantial homology requires only that the essential nature of the polypeptide, i.e. folding characteristics and unique features such as the leucine zipper are preserved. Thus, modifications of the leucine-zipper protein kinase are anticipated and are within the scope of the present invention. These modification may be deliberate, as through site directed mutagenesis, or may be accidental as through mutations in host which are producers of the protein. In some embodiments of the present invention polypeptides of the present invention may be at least about 75% homologous to the sequence set forth in SEQ ID NO: 2. In other embodiments of the present invention polypeptides may be at least about 85% homologous to the sequence set

forth in SEQ ID NO: 2. In yet other embodiments of the present invention polypeptides may be at least about 95% homologous to the sequence set forth in SEQ ID NO: 2. It is also anticipated that certain non-commonly occurring amino acids may be substituted for commonly occurring counterparts to confer desirable characteristics to the resulting polypeptide.

Furthermore, it is contemplated in some aspects of the present invention that a polypeptide may comprise only a portion of the sequence set forth in SEQ ID NO: 2. This may be the case, for example, for a chimeric protein encompassing active or otherwise desirable portions of a number of proteins. A portion may also refer to a truncated polypeptide, be it substantially truncated or only slightly truncated. Such truncated polypeptides may be the result of an idiosyncracy in the mode of production which results in truncation of amino acids from a terminal end, or a finding that the truncated polypeptide works as well or better than the full-length protein. For example, it might be found that the region directly surrounding the protein kinase domain at amino acids 231-243 is especially active.

Of course, in still other aspects of the present invention, the full-length protein, as set forth in SEQ ID NO: 2, is contemplated.

The leucine-zipper protein kinase of the present invention, depending on the pH of its environment, if suspended or in solution, or of its environment when crystallized or precipitated, if in solid form, may be in the form of pharmaceutically acceptable salts or may be in neutral form. The free amino acid groups of the protein are, of course, capable of forming acid addition salts with, for example, organic acids such as hydrochloric, phosphoric, or sulfuric acid; or with organic acids such as, for example, acetic, glycolic, succinic, or mandelic acid. The free carboxyl groups are capable of forming salts with bases, including inorganic bases such as sodium, potassium, or calcium hydroxides, and such organic bases as piperidine,

glucosamine, trimethylamine, choline, and caffeine. In addition, the protein may be modified by combination with other biological materials such as lipids and saccharides, or by side chain modifications such as acetylation of amino groups, phosphorylation of hydroxyl side chains or oxidation of sulfhydryl groups.

The leucine-zipper protein kinase is preferably purified and isolated. "Purified" and "isolated" as the terms are used herein, are meant to refer to molecules which have been purified or synthesized so as to be substantially homogenous. The terms do not exclude the possibility that certain impurities may be present in the composition as long as the essential nature of the protein is intact.

Tissue distribution analysis indicated that leucine-zipper protein kinase is present in the brain, more so in the adult than in the fetal brain based upon the detection of a 3.4Kb mRNA transcript. A smaller mRNA transcript, about 3.2Kb was detected in kidney and skeletal muscle. Adult lung tissue expressed both transcripts at a very low level. In fetal tissue, the only definite transcript seen is in the brain. These results can be seen in Figure 2.

The cDNA sequence of a novel leucine-zipper protein kinase is also provided by the present invention. The cDNA has a long open reading frame encoding 859 amino acids. The methionine codon at nucleotides 99-101 matches Kozak's consensus sequence for the initiation of translation. Kozak *Nucleic Acid Res 9*: 5233-5252 (1981). The polyadenylation signal AATAAC was found at nucleotides 3347-3352. Wickens and Stephenson, *Science 226:* 1045-1051 (1984). The 5' cap site is CATCCG, 90 base pairs from the initiation start site.

Homology searches of leucine-zipper protein kinase with the nucleotide and amino acid databases showed no homology to any known protein kinase family. Leucine-zipper protein kinase is most similar to serine/threonine specific protein kinases. The leucine zipper protein kinase protein

is believed to be a "non-receptor type kinase" based on its lack of a transmembrane domain. The consensus sequences for the ATP-binding site, Gly-Xaa-Gly-Xaa-Xaa-Gly and Lys residues are found at positions 537-544 and 548, respectively. The protein kinase domain was found to be at position 231-243. Taylor, et al., *Annu. Rev. Cell Biol.*, *8*, 429-462 (1992). Two overlapping sites of leucine zipper motif (leucine at every seventh amino acid) occur at position 442-468. A putative endoplasmic reticulum-targeting sequence was located at residues 415-418 (Pelham, H.R.B., *Annu. Rev. Cell Biol.*, *5*, 1-23 (1989).

Comparison with other members of the family of protein kinases indicate that leucine-zipper protein kinase has a number of novel features. First, the glycine rich loop in leucine zipper protein kinase is present towards the C-terminus of the catalytic domain, whereas in other protein kinases it is present near the N-terminus.

Endoplasmic reticulum targeting sequences (REEL) have been identified in both soluble; Pelham, H.R.B., *Annu. Rev. Cell. Biol.*, *5*, 1-23 (1989); and transmembrane; Jackson, et al., *EMBO J.*, *9*, 3153-3162 (1990) endoplasmic reticulum proteins. A lysine rich motif at the cytoplasmically-exposed C-terminus of some transmembrane proteins was described which conferred endoplasmic reticulum localization, although a more complex retention signal at the C-terminus has also be postulated. Gabathuler and Kvist, *J. Cell Biol.*, *111*, 1803-1810 (1990). Leucine-zipper protein kinase contains an endoplasmic reticulum targeting sequence which is located from amino acid 415-416, rather than at the extreme C-terminus of the protein.

Leucine-zipper protein kinase is also unique in that it contains a leucine-zipper motif, a sequence in which leucines occur at every seventh amino acid. Leucine-zippers contribute to targeting of various proteins (eg. glucose transporters, Asano, et al., *J. Biol. Chem.*, *267*, 19636-19641 (1992)) and permit dimerization of various cytoplasmic hormone receptors and enzymes. Forman, et al., *Mol*

Endocrinol, 3, 1610-1626 (1989). Leucine zippers are also a common feature of protein transcription factors, where they permit homo- or heterodimerization resulting in tight binding to DNA strands.

A leucine-zipper motif has been reported only once previously in a protein kinase, the bovine cGMP-dependent protein kinase, which has a leucine-isoleucine zipper motif at its N-terminus. Wernet, et al., *FEBS, 251*, 191-196 (1989).

Leucine-zipper protein kinase can be routinely synthesized in substantially pure form by standard techniques well known in the art, such as commercially available peptide synthesizers and the like.

Additionally, leucine-zipper protein kinase can be efficiently prepared using any of numerous well known recombinant techniques such as those described in U.S. patent No. 4,677,063 which patent is incorporated by reference as if fully set forth herein. Briefly, most of the techniques which are used to transform cells, construct vectors, extract messenger RNA, prepare cDNA libraries, and the like are widely practiced in the art, and most practitioners are familiar with the standard resource materials which describe specific conditions and procedures. However, for convenience, the following paragraphs may serve as a guideline.

Procaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains may also be used, such as *bacilli*, for example *Bacillus subtilis*, various species of *Pseudomonas*, or other bacterial strains. In such procaryotic systems, plasmid vectors which contain replication sites and control sequences derived from a species compatible with the host are used. For example, *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species by Bolivar, et al, *Gene* (1977) 2:95. pBR322 contains genes for ampicillin and tetracycline resistance, and thus provides additional markers which can be either

retained or destroyed in constructing the desired vector. Commonly used procaryotic control sequences include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta-lactamase (penicillinase) and lactose (lac) promoter systems (Chang, et al., *Nature* (1977) 198:1056) and the tryptophan (trp) promoter system (Goeddel, et al. *Nucleic Acids Res* (1980) 8:4057) and the lambda derived P_L promoter and N-gene ribosome binding site (Shimatake, et al., *Nature* (1981) 292:128).

In addition to bacteria, eucaryotic microbes, such as yeast, may also be used as hosts. Laboratory strains of *Saccharomyces cerevisiae*, Baker's yeast, are most used although a number of other strains are commonly available. While vectors employing the 2 micron origin of replication are illustrated, Broach, J.R., *Meth Enz* (1983) 101:307, other plasmid vectors suitable for yeast expression are known (*see, for example*, Stinchcomb, et al., *Nature* (1979) 282:39, Tschempe, et al., *Gene* (1980)10:157 and Clark, L., et al., *Meth Enz* (1983) 101:300). Control sequences for yeast vectors include promoters for the synthesis of glycolytic enzymes (Hess, et al., *J Adv Enzyme Req* (1968) 7:149; Holland, et al. *Biochemistry* (1978) 17:4900). Additional promoters known in the art include the promoter for 3-phosphoglycerate kinase (Hitzeman, et al., *J Biol Chem* (1980) 255:2073), and those for other glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acidphosphatase, degradative enzymes associated with nitrogen metabolism, and enzymes responsible for maltose and galactose utilization

(Holland, ibid). It is also believed terminator sequences are desirable at the 3' end of the coding sequences. Such terminators are found in the 3' untranslated region following the coding sequences in yeast-derived genes. Many of the vectors illustrated contain control sequences derived from the enolase gene containing plasmid peno46 (Holland, M.J., et al., *J Biol Chem* (1981) 256:1385) or the LEU2 gene obtained from YEp13 (Broach, J., et al., *Gene* (1978) 8:121), however any vector containing a yeast compatible promoter, origin of replication and other control sequences is suitable.

It is also, of course, possible to express genes encoding polypeptides in eucaryotic host cell cultures derived from multicellular organisms. See, for example, *Tissue Cultures*, Academic Press, Cruz and Patterson, editors (1973). Useful host cell lines include VERO, HeLa cells, and Chinese hamster ovary (CHO) cells. Expression vectors for such cells ordinarily include promoters and control sequences compatible with mammalian cells such as, for example, the commonly used early and late promoters from Simian Virus 40 (SV 40) Fiers, et al., *Nature* (1978) 273:113), or other viral promoters such as those derived from polyoma, Adenovirus 2, bovine papilloma virus, or avian sarcoma viruses. General aspects of mammalian cell host system transformations have been described e.g. by Axel; U.S. Pat. No. 4,399,216. It now appears, also that "enhancer" regions are important in optimizing expression; these are, generally, sequences found upstream or downstream of the promoter region in non-coding DNA regions. Origins of replication may be obtained, if needed, from viral sources. However, integration into the chromosome is a common mechanism for DNA replication in eucaryotes. Plant cells are also now available as hosts, and control sequences compatible with plant cells such as the nopaline synthase promoter and polyadenylation signal sequences (Depicker, A., et al., *J Mol Appl Gen* (1982) 1:561) are available.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described by Cohen, S.N., *Proc Natl Acad Sci (USA)* (1972) 69:2110, or methods described in *Molecular Cloning: A Laboratory Manual (1988)* Cold Spring Harbor Press, could be used for procaryotes or other cells which contain substantial cell wall barriers. Infection with *Agrobacterium tumefaciens* (Shaw, C. H., et al., *Gene* (1983) 23:315) is believed useful for certain plant cells. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology* (1978) 52:546 can be used. Transformations into yeast can be carried out according to the method of Van Solingen, P., et al., *J Bact* (1977) 130:946 and Hsiao, C.L., et al., *Proc Natl Acad Sci (USA)* (1979) 76:3829.

cDNA or genomic libraries can be screened using the colony hybridization procedure. Generally, each microtiter plate is replicated onto duplicate nitrocellulose filter papers (S&S type BA-85) and colonies are allowed to grow at 37° C. for 14-16 hr on L agar containing 50 μg/ml Amp. The colonies are lysed and DNA fixed to the filter by sequential treatment for 5 min with 500 mM NaOH, 1.5M NaCl, and are washed twice for 5 min each time with 5x standard saline citrate (SSC). Filters are air dried and baked at 80° C. for 2 hr. The duplicate filters are prehybridized at 42° C. for 6-8 hr with 10 ml per filter of DNA hybridization buffer (5xSSC, pH 7.0 5x Denhardt's solution (polyvinylpyrrolidine, plus Ficoll and bovine serum albumin; 1x=0.02% of each), 50 mM sodium phosphate buffer at pH 7.0, 0.2% SDS, 20 μg/ml Poly U, and 50 μg/ml denatured salmon sperm DNA).

The samples can be hybridized with kinased probe under conditions which depend on the stringency desired. Typical moderately stringent conditions employ a temperature of 42° C. for 24-36 hr with 1-5 ml/filter of DNA hybridization buffer containing probe. For higher

stringencies high temperatures and shorter times are employed. Generally, the filters are washed four times for 30 min each time at 37° C. with 2xSSC, 0.2% SDS and 50 mM sodium phosphate buffer at pH 7, then are washed twice with 2xSSC and 0.2% SDS, air dried, and are autoradiographed at -70° C. for 2 to 3 days.

Construction of suitable vectors containing the desired coding and control sequences employs standard ligation and restriction techniques which are well understood in the art. Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and religated in the form desired.

Site specific DNA cleavage can be performed by treating the DNA with a suitable restriction enzyme (or enzymes) under conditions which are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes. See, e.g., New England Biolabs, Product Catalog. In general, about 1 μg of plasmid or DNA sequence is cleaved by one unit of enzyme in about 20 μl of buffer solution. Incubation times of about one hour to two hours at about 37° C. are workable, although variations can be tolerated. After each incubation, protein can be removed by extraction with phenol/chloroform, and may be followed by ether extraction, and the nucleic acid recovered from aqueous fractions by precipitation with ethanol followed by running over a Sephadex G-5 spin column. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations can be found in *Methods in Enzymology* (1980) 65:499-560.

Restriction cleaved fragments may be blunt ended by treating with the large fragment of *E. coli* DNA polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTPs) using incubation times of about 15 to 25 min at 20° to 25° C. in 50 mM Tris pH 7.6,

50 mM NaCl, 6 mM $MgCl_2$, 6 mM DTT and 5-10 μM dNTPs. The Klenow fragment fills in at 5' sticky ends but chews back protruding 3' single strands, even though the four dNTPs are present. If desired, selective repair can be performed by supplying only one of the, or selected, dNTPs within the limitations dictated by the nature of the sticky ends. After treatment with Klenow, the mixture is extracted with phenol/chloroform and ethanol precipitated followed by running over a Sephadex G-50 spin column. Treatment under appropriate conditions with S1 nuclease results in hydrolysis of any single-stranded portion.

Synthetic oligonucleotides can be prepared by the triester method of Metteucci, et al. (*J Am Chem Soc* (1981) 103:3185) or using commercially available automated oligonucleotide synthesizers. Kinasing of single strands prior to annealing or for labeling is achieved using an excess, e.g., approximately 10 units of polynucleotide kinase to 0.1 nmole substrate in the presence of 50 mM Tris, pH 7.6, 10 mM $MgCl_2$, 5 mM dithiothreitol, 1-2 Mm ATP, 1.7 pmoles γ^{32}P-ATP (2.9 mCi/mmole), 0.1 mM spermidine, 0.1 mM EDTA.

Ligations can be performed in 15-30 μl volumes under the following standard conditions and temperatures: 20 mM Tris-Cl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 33 μg/ml GSA, 10 mM-50 mM NaCl, and either 40 μM ATP, 0.01-0.02 (Weiss) units T4 DNA ligase at 0° C. (for "sticky end" ligation) or 1 mM ATP, 0.3-0.6 (Weiss) units T4 DNA ligase at 14° C. (for "blunt end" ligation). Intermolecular "sticky end" ligations are usually performed at 33-100 μg/ml total DNA concentrations (5-100 nM total end concentration). Intermolecular blunt end ligations (usually employing a 10-30 fold molar excess of linkers) are performed at 1 μM total ends concentration.

In vector construction employing "vector fragments", the vector fragment can be treated with bacterial alkaline phosphatase (BAP) in order to remove the 5' phosphate and prevent religation of the vector. BAP

digestions can be conducted at pH 8 in approximately 150 mM Tris, in the presence of Na+ and Mg^{+2} using about 1 unit of BAP per μg of vector at 60° C. for about one hour. In order to recover the nucleic acid fragments, the preparation is extracted with phenol/chloroform and ethanol precipitated and desalted by application to a Sephadex G-50 spin column. Alternatively, religation can be prevented in vectors which have been double digested by additional restriction enzyme digestion of the unwanted fragments.

For portions of vectors derived from cDNA or genomic DNA which require sequence modifications, site specific primer directed mutagenesis can be used. This is conducted using a primer synthetic oligonucleotide complementary to a single stranded phage DNA to be mutagenized except for limited mismatching, representing the desired mutation. Briefly, the synthetic oligonucleotide is used as a primer to direct synthesis of a strand complementary to the phage, and the resulting double-stranded DNA is transformed into a phage-supporting host bacterium. Cultures of the transformed bacteria are plated in top agar, permitting plaque formation from single cells which harbor the phage.

Theoretically, 50% of the new plaques will contain the phage having, as a single strand, the mutated form; 50% will have the original sequence. The resulting plaques can be hybridized with kinased synthetic primer at a temperature which permits hybridization of an exact match, but at which the mismatches with the original strand are sufficient to prevent hybridization. Plaques which hybridize with the probe are then picked, cultured, and the DNA recovered.

Correct ligations for plasmid construction can be confirmed by first transforming a suitable host with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or using other markers depending on the mode of plasmid construction, as is understood in the art. Plasmids from the transformants can then be prepared according to the

method of Clewell, D.B., et al. *Proc Natl Acad Sci (USA)* (1969) 62:1159, optionally following chloramphenicol amplification (Clewell, D.B., *J Bacteriol* (1972) 110:667). The isolated DNA Is analyzed by restriction and/or sequenced by the dideoxy method of Snager, F., et al. *Proc Natl Acad Sci (USA)* (1977) 74:5463 as further described by Messing, et al., F. Supp. *Nucleic Acids Res* (1981) 9.309, or by the method of Maxam, et al., *Methods in Enzymology* (1980) 65:499.

In accordance with the present invention polynucleotide probes specifically hybridizable to a portion of the leucine zipper protein kinase gene are provided. Polynucleotide probes substantially homologous to a portion of the leucine-zipper protein kinase gene are also provided. Such probes may be used for diagnostic or research purposes to detect or quantitate the expression of leucine zipper protein kinase in a sample such as by detecting the presence or absence of polynucleotide duplex formation between the polynucleotide probe and leucine-zipper protein kinase gene. Samples may derived from cell culture or may be derived from a patient. Samples may be biological fluids such as synovial fluid in some aspects of the invention. Tissue samples may also be used in some embodiments of the present invention. Detection of the presence of polynucleotide duplexes is indicative of the presence of the leucine-zipper protein kinase gene in a sample and may be indicative of diseases associated with leucine zipper protein kinase, such as tumors of the central nervous system. Provision of means for detecting hybridization of polynucleotides with the leucine-zipper protein kinase gene can routinely be accomplished. Such provision may include enzyme conjugation, radiolabelling or any other suitable detection systems. Kits for detecting the presence or absence of leucine zipper protein kinase or a particular transcript thereof may also be prepared. Said polynucleotide probes may range in length from about 5 to about 100 nucleotide units. In more preferred embodiments of the present invention the

probes may be from about 8 to about 75 nucleotide units in length. Ideally, said probes range in length from about 12 to about 50 nucleotide units. It is recognized that since polynucleotide probes of the present invention may preferably not exceed 100 nucleotides in length, said probes may specifically hybridize to only a portion of the targeted sequence. The portion of the leucine zipper protein kinase sequence to be targeted can be identified by one skilled in the art. Most suitably, a target sequence is chosen which is unique, thereby decreasing background noise attributable to hybridization by the probe other than to the target. By way of example, one skilled in the art would be unlikely to select a repeating sequence of adenine nucleotide units as this is a common sequence occurring in many genes. The practitioner might choose to perform a search and comparison of sequences found in a sequence repository such as Genbank in order to identify and design a useful probe. Such methods of conventionally used to identify unique sequences. These unique sequences, when used as probes, need not necessarily be crucial to the regulation of the expression of leucine-zipper protein kinase.

In accordance with other methods of the present invention, neuronal cells may be contacted with leucine-zipper protein kinase, or a portion thereof in order to inhibit cellular proliferation. While not wishing to be bound to a particular theory, it is believed that the addition of exogenous leucine-zipper protein kinase, or portions thereof may interfere with specific protein-protein or protein-nucleic acid interactions involved in cellular hyperproliferation. For example, by administering an inactive leucine-zipper protein kinase polypeptide or a portion thereof, it may be possible to compete with naturally occurring leucine-zipper protein kinase for binding regions of target nucleic acid molecules or polypeptides in order to modulate its effect in the cell at the level of protein-protein or protein-nucleic acid interactions. In this way, it may be possible to treat a

mammal suffering from tumors of the central nervous system by inhibiting the overexpression of leucine-zipper protein kinase *in vivo* or by interfering with a vital signal in the chain of signals leading to tumorigenicity.

For methods of the present invention, leucine-zipper protein kinase may be formulated into pharmacological compositions containing an effective amount of leucine-zipper protein kinase and a usual nontoxic carrier, such carriers being known to those skilled in the art. The compositions may be administered by a method suited to the form of the composition. Such compositions are, for example, in the form of usual liquid preparations including solutions, suspensions, emulsions, and the like which can be given orally, intravenously, subcutaneously or intramuscularly.

The present invention is also directed to methods of inhibiting hyperproliferation of neuronal cells comprising contacting the cells with oligonucleotides substantially complementary to a portion of the nucleic acid sequence set forth in SEQ ID NO: 1. "Complementary" in the context of this invention, means the ability to form hydrogen bonds, also known as Watson-Crick base pairing, between complementary bases, usually on opposite nucleic acid strands or two regions of a nucleic acid strand, to form a double-stranded duplex. Guanine and cytosine are examples of complementary bases which are known to form three hydrogen bonds between them. Adenine and thymine are examples of complementary bases which are known to form two hydrogen bonds between them. "Specifically hybridizable" and "substantially complementary" are terms which indicate a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide (or polynucleotide probe) to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays and therapeutic treatment, or, in the case of *in vitro* assays, under conditions in which the assays are conducted. It is understood that an

oligonucleotide, polynucleotide probe or nucleic acid sequence need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable or effective in methods of the present invention. Thus, in some embodiments of the present invention abot 50% homology is envisioned. About 75% percent homology is preferred in some aspects of the present invention and about 80% homology is still more preferred. Ninety percent homology may be preferred in yet other embodiments of the present invention. In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid. This term includes oligomers consisting of naturally occurring bases, sugars and intersugar (backbone) linkages as well as oligomers having non-naturally occurring portions which function similarly. The oligonucleotides in accordance with this invention preferably comprise from about 5 to about 50 nucleotide units. It is more preferred that such oligonucleotides comprise from about 8 to 30 nucleotide units, and still more preferred to have from about 12 to 25 nucleotide units. Oligonucleotides of the present invention may be prepared by standard techniques such as solid-phase synthesis which are well known to those skilled in the art.

Furthermore, in accordance with methods of the present invention, a therapeutically effective amount of oligonucleotide is administered to a mammal suffering from tumors of the central nervous system.

Oligonucleotides may be formulated in a pharmaceutical composition, which may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the oligonucleotide. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like in addition to oligonucleotides.

The pharmaceutical compositions of the present invention may be administered in a number of ways depending

on whether local or systemic treatment is desired, and on the area to be treated. Administration may be done topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip or subcutaneous, intraperitoneal or intramuscular injection.

Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms or gloves may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

Dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

The following examples are illustrative and are not meant to be limiting of the present invention.

Examples

Example 1

Cells

Human teratocarcinoma line NT2 was differentiated into postmitotic neurons NT2-N with retinoic acid as previously described (Pleasure et al., *J. Neurosci 12*: 1802-1815 (1992); Younkin et al., *Proc. Natl. Acad. Sci. USA 90*:

2174-2178 (1992)). Poly(A)$^+$ RNA was isolated from both NT2 and NT2-N neurons using Invitrogen mRNA kit.

Example 2

Subtractive Hybridization and DNA Amplification

Invitrogen's Subtractor probe kit was used according to the manufacturer's instructions to isolate two different subtracted cDNAs UND and DIFF. UND was enriched in transcripts expressed in the undifferentiated stage whereas DIFF was enriched in transcripts present in the neurons. One μg portions of UND and DIFF mRNA were used for PCT amplification with degenerate primers as described in Wilks *Proc. Natl. Acad. Sci. USA 86*: 1603-1607 (1989). PCT was performed using a Geneamp kit (Cetus) with 1μg of each of the degenerate primers. The final concentration of magnesium was 2.1 mM. PCT cycling was performed on a Perkin-Elmer 480 thermal cycler for 39 cycles with a profile of 1.3 minutes at 95°C (denaturation), 2 minutes at 45°C (annealing), and 2 minutes at 64°C (elongation).

Example 3

Subcloning of Amplified DNAs and DNA Sequencing

The PCR reaction mixture were run on 4% Nusieve agarose gel and the amplified band of ~220 bp was excised. The band was purified using Magic PCR Kit (Promega). The amplified DNA was digested with the restriction enzymes BamH1/EcoR1. The amplified DNAs were subcloned into the BamH1 and EcoR1 cleaved Bluescript DNA. A total of about 200 clones (100 representing UND and 100 representing DIFF) were examined by sequencing using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems). Plasmid DNA was isolated using Qiagen column 20. The cycle sequencing reactions were performed in a Perkin-Elmer 480 thermal cycler for 25 cycles with a profile of 96°C for 30 seconds, 40°C for 15 seconds, and 60°C for 4 minutes. Following separation of the extension products on a Select-D G-50 column (5 Prime 3 Prime) the reaction mixtures were dried,

resuspended in 4μl of 5:1 formamide/50 mM EDTA, loaded on a 6% sequencing gel, and analyzed using an Applied Biosystems 373 fluorescent sequencer.

Example 4

cDNA Library Screening

The 210 bp 10.2 PCR clone from undifferentiated clones was radiolabelled with [^{32}P]dCTP and used to probe ~10^6 plaques from an amplified human fetal brain library (Stratagene) to obtain larger cDNA clones. Hybridization was carried out overnight at 42°C in 50% Formamide, 5xSSPE, 5xDenhardt's, 1% SDS, 100ug/ml sheared salmon sperm DNA, and $1x10^6$ cpm/ml of probe. Filters were washed at 60°C twice in 2xSSC containing 0.1% SDS, and exposed overnight to Kodak XAR-5 film at -70°C.

Example 5

Sequence Determination

cDNAs were subcloned into a plasmid vector BluescriptSk. For complete sequence determination, unidirectional nested deletions was performed using the Exo111/Mung Bean nuclease kit from Stratagene. The colonies obtained after deletions were sequenced as described earlier using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems). The DNA sequence obtained was determined after sequencing twice.

Example 6

Sequence comparisons

All sequence manipulations were done on a VAX using the University of Wisconsin Genetics Computer Group Sequence Analysis Software Package. DNA fragments obtained after nested deletions was assembled into Contigs using the Programme Sequencer 2.0 (Gene Codes Corp). Protein analysis was done using MacVector (IBI).

Example 7

RNA Analysis

Human multiple tissue northern blots were purchased from Clontech laboratories. Hybridization conditions were similar to that used for library screening. Filters were washed to a final stringency of 0.1 x SSC/0.1% SDS at 65°C before exposure to XAR-5 x-ray film.

Example 8

Localization of the *zpk* Gene to Chromosome 12

Fluorescence *in situ* hybridization (FISH) and somatic cell hybrid analysis were used to localize the *zpk* gene to chromosome 12 of the human genome. Briefly, a *zpk* clone, ZPK P1, was obtained using two oligonucleotides CCATGAAAGCCACTCGTATTCCT (SEQ ID NO:3) and TCTTTCTGTTGATTATGTGGCGC (SEQ ID NO:4), both of which correspond to the 3' untranslated region. This clone, ZPK P1, was shown to contain the *zpk* sequence by digesting the plasmid with EcoR1 and HindIII and by Southern analysis with [α-^{32}P]-labeled ZPK cDNA.

To map the *zpk* gene, FISH and somatic cell hybrid analysis were performed. The ZPK P1 clone was labeled by nick-translation with digoxigenin-11-dUTP using a large fragment labeling kit from Oncor (Gaithersburg, MD). Human metaphase chromosome spreads were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes from normal controls according to routine methods. Labeled ZPK (200 ng) and Cot-1 DNA (20 μg) were dissolved in 15 μl of Hybrisol VII (Oncor), denatured for 5 minutes at 70°C, preannealed at 37°C for 30 minutes, and applied to slides that had been denatured at 70°C for 2 minutes. The slides were hybridized overnight at 37°C and then rinsed at 39°C in 50% formamide/1X SSC for 10 minutes, followed by two washes in 2X SSC for 2 minutes each. Detection of probes was accomplished with anti-digoxigen rhodamine (Oncor). Cells were counterstained with DAPI/antifade (Oncor) and viewed with a Zeiss axiophot microscope equipped with a triple band

pass filter set. A total of 50 metaphase cells were examined in replicate experiments and demonstrated signals on one or both chromatids for both chromosomes 12 in all metaphases examined.

To confirm independently the map location for *zpk* to chromosome 12, ZPK cDNA was hybridized to a somatic cell hybrid mapping panel. The panel consisted of the following cell lines and chromosomal number (in parenthesis): GM10880 (1, 3, 14); GM10826B (2); GM10253 (3); GM10115 (4); GM10114 (5); GM10629 (6); GM10791 (7); GM10156B (8); GM10611 (9); GM10926B (10); GM10927A9 (11); GM10868 (12); GM10898 (13); GM10479 (14); GM11418 (15); GM10567 (16); GM10498 (17); GM11010 (18); GM10612 (19); GM10478 (4,20); GM08854 (21); GM10888 (22); PHL-17 (X); and GM06317 (Y). The mouse cell line Cl 1-D and chinese hamster cell line RJK-88 were used as rodent controls.

Ten micrograms of DNA from each cell line was digested with HindIII, loaded onto a 0.8% agarose gel, subjected to electrophoresis, and blotted onto Hybond-N (Amersham). The ZPK cDNA probe was labeled by the random primer method with [α-^{32}P] and hybridized overnight at 68°C in Church's solution (1 mM EDTA, 7% SDS, 0.5 M sodium phosphate, pH 7.2). Blots were washed for one hour in 2X SSC/0.1% SDS at 68°C and exposed to radiography film for 48 hours. Two bands corresponding to those seen on Southern blots from normal human genomic DNA were observed in GM10868, which contains chromosome 12 as the only human chromosome, confirming that the human *zpk* gene maps to chromosome 12.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Usharani R. Reddy, David Pleasure and the Children's Hospital of Philadelphia

(ii) TITLE OF INVENTION: Novel Protein Kinase, Nucleic Acid Sequences Encoding the Same and Methods Related Thereto

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch disk, 720 Kb
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WordPerfect 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: N/A
(B) FILING DATE: herewith
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/205,018
(B) FILING DATE: 01-MAR-1994

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 35,152
(C) REFERENCE/DOCKET NUMBER: CH-0488

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3389 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 99..2105
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AGCATCCGGA GCGGAGCTGC AGCAGCGCCG CCTTTTGTGC TGCGGCCGCG GAGCCCCCGA 60

GGGCCCAGTG TTCACCATCA TACCAGGGGC CAGAGGCG ATG GCT TGC CTC CAT     113
                                          Met Ala Cys Leu His
                                            1               5

GAG ACC CGA ACA CCC TCT CCT TCC TTT GGG GGC TTT GTG TCT ACC CTA   161
Glu Thr Arg Thr Pro Ser Pro Ser Phe Gly Gly Phe Val Ser Thr Leu
                 10                  15                  20
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AGT GAG GCA TCC ATG CGC AAG CTG GAC CCA GAC ACT TCT GAC TGC ACT 209
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25 30 35

CCC GAG AAG GAC CTG ACG CCT ACC CAT GTC CTG CAG CTA CAT GAG CAG 257
Pro Glu Lys Asp Leu Thr Pro Thr His Val Leu Gln Leu His Glu Gln
40 45 50

GAT GCA GGG GGC CCA GGG GGA GCA GCT GGG TCA CCT GAG AGT CGG GCA 305
Asp Ala Gly Gly Pro Gly Gly Ala Ala Gly Ser Pro Glu Ser Arg Ala
55 60 65

TCC AGA GTT CGA GCT GAC GAG GTG CGA CTG CAG TGC CAG AGT GGC AGT 353
Ser Arg Val Arg Ala Asp Glu Val Arg Leu Gln Cys Gln Ser Gly Ser
70 75 80 85

GGC TTC CTT GAG GGC CTC TTT GGC TGC CTG CGC CCT GTC TGG ACC ATG 401
Gly Phe Leu Glu Gly Leu Phe Gly Cys Leu Arg Pro Val Trp Thr Met
90 95 100

ATT GGC AAA GCC TAC TCC ACT GAG CAC AAG CAG CAG CAG GAA GAC CTT 449
Ile Gly Lys Ala Tyr Ser Thr Glu His Lys Gln Gln Gln Glu Asp Leu
105 110 115

TGG GAG GTC CCC TTT GAG GAA ATC CTG GAC CTG CAG TGG GTG GGC TCA 497
Trp Glu Val Pro Phe Glu Glu Ile Leu Asp Leu Gln Trp Val Gly Ser
120 125 130

GGG GCC CAG GGT GCT GTC TTC CTG GGG CGC TTC CAC GGG GAG GAG GTG 545
Gly Ala Gln Gly Ala Val Phe Leu Gly Arg Phe His Gly Glu Glu Val
135 140 145

GCT GTG AAG AAG GTG CGA GAC CTC AAA GAA ACC GAC ATC AAG CAC TTG 593
Ala Val Lys Lys Val Arg Asp Leu Lys Glu Thr Asp Ile Lys His Leu
150 155 160 165

CGA AAG CTG AAG CAC CCC AAC ATC ATC ACT TTC AAG GGT GTG TGC ACC 641
Arg Lys Leu Lys His Pro Asn Ile Ile Thr Phe Lys Gly Val Cys Thr
170 175 180

CAG GCT CCC TGC TAC TGC ATC CTC ATG GAG TTC TGC GCC CAG GGC CAG 689
Gln Ala Pro Cys Tyr Cys Ile Leu Met Glu Phe Cys Ala Gln Gly Gln
185 190 195

CTG TAT GAG GTA CTG CGG GCT GGC CGC CCT GTC ACC CCC TCC TTA CTG 737
Leu Tyr Glu Val Leu Arg Ala Gly Arg Pro Val Thr Pro Ser Leu Leu
200 205 210

GTT GAC TGG TCC ATG GGC ATC GCT GGT GGC ATG AAC TAC CTG CAC CTG 785
Val Asp Trp Ser Met Gly Ile Ala Gly Gly Met Asn Tyr Leu His Leu
215 220 225

CAC AAG ATT ATC CAC AGG GAT CTC AAG TCA CCC AAC ATG CTA ATC ACC 833
His Lys Ile Ile His Arg Asp Leu Lys Ser Pro Asn Met Leu Ile Thr
230 235 240 245

TAC GAC GAT GTG GTG AAG ATC TCA GAT TTT GGC ACT TCC AAG GAG CTG 881
Tyr Asp Asp Val Val Lys Ile Ser Asp Phe Gly Thr Ser Lys Glu Leu
250 255 260

AGT GAC AAG AGC ACC AAG ATG TCC TTT GCA GGG ACA GTA GCC TGG ATG 929
Ser Asp Lys Ser Thr Lys Met Ser Phe Ala Gly Thr Val Ala Trp Met
265 270 275

GCC CCT GAG GTG ATC CGC AAT GAA CCT GTG TCT GAG AAG GTC GAC ATC 977
Ala Pro Glu Val Ile Arg Asn Glu Pro Val Ser Glu Lys Val Asp Ile
280 285 290

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TGG TCC TTT GGC GTG GTG CTA TGG GAA CTG CTG ACT GGT GAG ATC CCC   1025
Trp Ser Phe Gly Val Val Leu Trp Glu Leu Leu Thr Gly Glu Ile Pro
    295                 300                 305

TAC AAA GAC GTA GAT TCC TCA GCC ATT ATC TGG GGT GTG GGA AGC AAC   1073
Tyr Lys Asp Val Asp Ser Ser Ala Ile Ile Trp Gly Val Gly Ser Asn
310                 315                 320                 325

AGT CTC CAT CTG CCC GTG CCC TCC AGT TGC CCA GAT GGT TTC AAG ATC   1121
Ser Leu His Leu Pro Val Pro Ser Ser Cys Pro Asp Gly Phe Lys Ile
                330                 335                 340

CTG CTT CGC CAG TGC TGG AAT AGC AAA CCA CGA AAT CGC CCA TCA TTC   1169
Leu Leu Arg Gln Cys Trp Asn Ser Lys Pro Arg Asn Arg Pro Ser Phe
            345                 350                 355

CGA CAG ATC CTG CTG CAT CTG GAC ATT GCC TCA GCT GAT GTA CTC TCC   1217
Arg Gln Ile Leu Leu His Leu Asp Ile Ala Ser Ala Asp Val Leu Ser
        360                 365                 370

ACA CCC CAG GAG ACT TAC TTT AAG TCC CAG GCA GAG TGG CGG GAA GAA   1265
Thr Pro Gln Glu Thr Tyr Phe Lys Ser Gln Ala Glu Trp Arg Glu Glu
    375                 380                 385

GTA AAA CTG CAC TTT GAA AAG ATT AAG TCA GAA GGG ACC TGT CTG CAC   1313
Val Lys Leu His Phe Glu Lys Ile Lys Ser Glu Gly Thr Cys Leu His
390                 395                 400                 405

CGC CTA GAA GAG GAA CTG GTG ATG AGG AGG AGG GAG GAG CTC AGA CAC   1361
Arg Leu Glu Glu Glu Leu Val Met Arg Arg Arg Glu Glu Leu Arg His
                410                 415                 420

GCC CTG GAC ATC AGG GAG CAC TAT GAA AGG AAG CTG GAG AGA GCC AAC   1409
Ala Leu Asp Ile Arg Glu His Tyr Glu Arg Lys Leu Glu Arg Ala Asn
            425                 430                 435

AAC CTG TAT ATG GAA CTT AAT GCC CTC ATG TTG CAG CTG GAA CTC AAG   1457
Asn Leu Tyr Met Glu Leu Asn Ala Leu Met Leu Gln Leu Glu Leu Lys
        440                 445                 450

GAG AGG GAG CTG CTC AGG CGA GAG CAA GCT TTA GAG CGG AGG TGC CCA   1505
Glu Arg Glu Leu Leu Arg Arg Glu Gln Ala Leu Glu Arg Arg Cys Pro
    455                 460                 465

GGC CTG CTG AAG CCA CAC CCT TCC CGG GGC CTC CTG CAT GGA AAC ACA   1553
Gly Leu Leu Lys Pro His Pro Ser Arg Gly Leu Leu His Gly Asn Thr
470                 475                 480                 485

ATG GAG AAG CTT ATC AAG AAG AGG AAT GTG CCA CAG AAT CTG TCA CCC   1601
Met Glu Lys Leu Ile Lys Lys Arg Asn Val Pro Gln Asn Leu Ser Pro
                490                 495                 500

CAT AGC CAA AGG CCA GAT ATC CTC AAG GCG GAG TCT TTG CTC CCT AAA   1649
His Ser Gln Arg Pro Asp Ile Leu Lys Ala Glu Ser Leu Leu Pro Lys
            505                 510                 515

CTA GAT GCA GCC CTG AGT GGG GTG GGG CTT CCT GGG TGT CCT AAG GCC   1697
Leu Asp Ala Ala Leu Ser Gly Val Gly Leu Pro Gly Cys Pro Lys Ala
        520                 525                 530

CCC CCC TCA CCA GGA CGG AGT CGC CGT GGC AAG ACC CGT CAC CGC AAG   1745
Pro Pro Ser Pro Gly Arg Ser Arg Arg Gly Lys Thr Arg His Arg Lys
    535                 540                 545

GCC AGC GCC AAG GGG AGC TGT GGG GAC CTG CCT GGG CTT CGT ACA GCT   1793
Ala Ser Ala Lys Gly Ser Cys Gly Asp Leu Pro Gly Leu Arg Thr Ala
550                 555                 560                 565
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GTG CCA CCC CAT GAA CCT GGA GGA CCA GGA AGC CCA GGG GGC CTA GGA  1841
Val Pro Pro His Glu Pro Gly Gly Pro Gly Ser Pro Gly Gly Leu Gly
                570                 575                 580

GGG GGA CCC TCA GCC TGG GAG GCC TGC CCT CCC GCC CTC CGT GGG CTT  1889
Gly Gly Pro Ser Ala Trp Glu Ala Cys Pro Pro Ala Leu Arg Gly Leu
            585                 590                 595

CAT CAT GAC CTC CTG CTC CGC AAA ATG TCT TCA TCG TCC CCA GAC CTG  1937
His His Asp Leu Leu Leu Arg Lys Met Ser Ser Ser Ser Pro Asp Leu
        600                 605                 610

CTG TCA GCA GCA CTA GGG TCC CGG GGC CGG GGG GCC ACA GGC GGA GCT  1985
Leu Ser Ala Ala Leu Gly Ser Arg Gly Arg Gly Ala Thr Gly Gly Ala
    615                 620                 625

GGG GAT CCT GGC TCA CCA CCT CCG GCC CGG GGT GAC ACC CCA CCA AGT  2033
Gly Asp Pro Gly Ser Pro Pro Pro Ala Arg Gly Asp Thr Pro Pro Ser
630                 635                 640                 645

GAG GGC TCA GCC CCT GGC TCC ACC AGC CCA GAT TCA CCT GGG GGA GCC  2081
Glu Gly Ser Ala Pro Gly Ser Thr Ser Pro Asp Ser Pro Gly Gly Ala
                650                 655                 660

AAA GGG GAA CCA CCT CCT CCA GTA GGG CCT GGT GAA GGT GTG GGG CTT  2129
Lys Gly Glu Pro Pro Pro Pro Val Gly Pro Gly Glu Gly Val Gly Leu
            665                 670                 675

CTG GGA ACT GGA AGG GAA GGG ACC TCA GGC CGG GGA GGA AGC CGG GCT  2177
Leu Gly Thr Gly Arg Glu Gly Thr Ser Gly Arg Gly Gly Ser Arg Ala
        680                 685                 690

GGG TCC CAG CAC TTG ACC CCA TCT GCA CTG CTG TAC AGG GCT GCC GTC  2225
Gly Ser Gln His Leu Thr Pro Ala Ala Leu Leu Tyr Arg Ala Ala Val
    695                 700                 705

ACC CGA AGT CAG AAA CGT GGC ATC TCA TCG GAA GAG GAG GAA GGA GAG  2273
Thr Arg Ser Gln Lys Arg Gly Ile Ser Ser Glu Glu Glu Glu Gly Glu
710                 715                 720                 725

GTA GAC AGT GAA GTA GAG CTG ACA TCA AGC CAG AGG TGG CCT CAG AGC  2321
Val Asp Ser Glu Val Glu Leu Thr Ser Ser Gln Arg Trp Pro Gln Ser
                730                 735                 740

CTG AAC ATG CGC CAG TCA CTA TCT ACC TTC AGC TCA GAG AAT CCA TCA  2369
Leu Asn Met Arg Gln Ser Leu Ser Thr Phe Ser Ser Glu Asn Pro Ser
            745                 750                 755

GAT GGG GAG GAA GGC ACA GCT AGT GAA CCT TCC CCC AGT GGC ACA CCT  2417
Asp Gly Glu Glu Gly Thr Ala Ser Glu Pro Ser Pro Ser Gly Thr Pro
        760                 765                 770

GAA GTT GGC AGC ACC AAC ACT GAT GAG CGG CCA GAT GAG CGG TCT GAT  2465
Glu Val Gly Ser Thr Asn Thr Asp Glu Arg Pro Asp Glu Arg Ser Asp
    775                 780                 785

GAC ATG TGC TCC CAG GGC TCA GAA ATC CCA CTG GAC CCA CCT CCT TCA  2513
Asp Met Cys Ser Gln Gly Ser Glu Ile Pro Leu Asp Pro Pro Pro Ser
790                 795                 800                 805

GAG GTC ATC CCT GGC CCT GAA CCC AGC TCC CTG CCC ATT CCA CAC CAG  2561
Glu Val Ile Pro Gly Pro Glu Pro Ser Ser Leu Pro Ile Pro His Gln
                810                 815                 820

GAA CTT CTC AGA GAG CGG GGC CCT CCC AAT TCT GAG GAC TCA GAC TGT  2609
Glu Leu Leu Arg Glu Arg Gly Pro Pro Asn Ser Glu Asp Ser Asp Cys
            825                 830                 835
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GAC AGC ACT GAA TTG GAC AAC TCC AAC AGC GTT GAT GCC TTG CGC CCC   2657
Asp Ser Thr Glu Leu Asp Asn Ser Asn Ser Val Asp Ala Leu Arg Pro
        840                 845                 850

CCA GCT TCC CTC CCT CCA TGAAAGCCAC TCGTATTCCT TGTACATAGA          2705
Pro Ala Ser Leu Pro Pro
    855

GAAATATTTA TATGGATTAT ATATATATAC ATATATATAT ATATATGCGC CACATAATCA 2765

ACAGAAAGAT GGGGCTGTCC CAGCCGTAAG TCAGGCTCGA GGGAGACTGA TCCCCTGACC 2825

AATTCACCTG ATAAACTCTA GGGACACTGG CAGCTGTGGA AATGAATGAG GCACAGCCGT 2885

AGAGCTGTGG CTAAGGGCAA GCCCCTTCCT GCCCCACCCC ATTCCTTATA TTCAGCAAGC 2945

AACAAGGCAA TAGAAAAGCC AGGGTTGTCT TTATATTCTT TATCCCCAAA TAATAGGGGG 3005

TGGGGGGAGG GGCGGTGGGA GGGGCAGGAG AGAAAACCAC TTAGACTGCA CTTTTCTGTT 3065

CCGTTTACTC TGTTTACACA TTTTGCACTT GGGAGGAGGG AGGCTAAGGC TGGGTCCTCC 3125

CCTCTGAGGT TTCTCAGGTG GCAATGTAAC TCATTTTTTT GTCCCACCAT TTATCTTCTC 3185

TGCCCAAGCC CTGTCTTAAG GCCCAGGGGG AGGTTAGGAG ACTGATAGCA TGTGATGGCT 3245

CAGGCTGAAG AACCGGGGTT CTGTTTAAGT CCCTGCTTTT ATCCTGGTGC CTGATTGGGG 3305

TGGGGACTGT CCTACTGTAA CCCCTGTGAA AAACCTTGAA AAATAACACT CCATGCAGGA 3365

AAAAAAAAAA AAAAAAAAAA AAAA                                        3389
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 859 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Gly Phe Val Ser Thr Leu Ser Glu Ala Ser Met Arg Lys Leu Asp
                20                  25                  30

Pro Asp Thr Ser Asp Cys Thr Pro Glu Lys Asp Leu Thr Pro Thr
                35                  40                  45

His Val Leu Gln Leu His Glu Gln Asp Ala Gly Gly Pro Gly Gly
                50                  55                  60

Ala Ala Gly Ser Pro Glu Ser Arg Ala Ser Arg Val Arg Ala Asp
                65                  70                  75

Glu Val Arg Leu Gln Cys Gln Ser Gly Ser Gly Phe Leu Glu Gly
                80                  85                  90

Leu Phe Gly Cys Leu Arg Pro Val Trp Thr Met Ile Gly Lys Ala
                95                  100                 105

Tyr Ser Thr Glu His Lys Gln Gln Gln Glu Asp Leu Trp Glu Val
                110                 115                 120
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Pro Phe Glu Glu Ile Leu Asp Leu Gln Trp Val Gly Ser Gly Ala
125 130 135

Gln Gly Ala Val Phe Leu Gly Arg Phe His Gly Glu Glu Val Ala
140 145 150

Val Lys Lys Val Arg Asp Leu Lys Glu Thr Asp Ile Lys His Leu
155 160 165

Arg Lys Leu Lys His Pro Asn Ile Ile Thr Phe Lys Gly Val Cys
170 175 180

Thr Gln Ala Pro Cys Tyr Cys Ile Leu Met Glu Phe Cys Ala Gln
185 190 195

Gly Gln Leu Tyr Glu Val Leu Arg Ala Gly Arg Pro Val Thr Pro
200 205 210

Ser Leu Leu Val Asp Trp Ser Met Gly Ile Ala Gly Gly Met Asn
215 220 225

Tyr Leu His Leu His Lys Ile Ile His Arg Asp Leu Lys Ser Pro
230 235 240

Asn Met Leu Ile Thr Tyr Asp Asp Val Val Lys Ile Ser Asp Phe
245 250 255

Gly Thr Ser Lys Glu Leu Ser Asp Lys Ser Thr Lys Met Ser Phe
260 265 270

Ala Gly Thr Val Ala Trp Met Ala Pro Glu Val Ile Arg Asn Glu
275 280 285

Pro Val Ser Glu Lys Val Asp Ile Trp Ser Phe Gly Val Val Leu
290 295 300

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305 310 315

Ser Ala Ile Ile Trp Gly Val Gly Ser Asn Ser Leu His Leu Pro
320 325 330

Val Pro Ser Ser Cys Pro Asp Gly Phe Lys Ile Leu Leu Arg Gln
335 340 345

Cys Trp Asn Ser Lys Pro Arg Asn Arg Pro Ser Phe Arg Gln Ile
350 355 360

Leu Leu His Leu Asp Ile Ala Ser Ala Asp Val Leu Ser Thr Pro
365 370 375

Gln Glu Thr Tyr Phe Lys Ser Gln Ala Glu Trp Arg Glu Glu Val
380 385 390

Lys Leu His Phe Glu Lys Ile Lys Ser Glu Gly Thr Cys Leu His
395 400 405

Arg Leu Glu Glu Glu Leu Val Met Arg Arg Arg Glu Glu Leu Arg
410 415 420

His Ala Leu Asp Ile Arg Glu His Tyr Glu Arg Lys Leu Glu Arg
425 430 435

Ala Asn Asn Leu Tyr Met Glu Leu Asn Ala Leu Met Leu Gln Leu
440 445 450

Glu Leu Lys Glu Arg Glu Leu Leu Arg Arg Glu Gln Ala Leu Glu
455 460 465

Arg Arg Cys Pro Gly Leu Leu Lys Pro His Pro Ser Arg Gly Leu
470 475 480

Leu His Gly Asn Thr Met Glu Lys Leu Ile Lys Lys Arg Asn Val
485 490 495

Pro Gln Asn Leu Ser Pro His Ser Gln Arg Pro Asp Ile Leu Lys
500 505 510

Ala Glu Ser Leu Leu Pro Lys Leu Asp Ala Ala Leu Ser Gly Val
515 520 525

Gly Leu Pro Gly Cys Pro Lys Ala Pro Pro Ser Pro Gly Arg Ser
530 535 540

Arg Arg Gly Lys Thr Arg His Arg Lys Ala Ser Ala Lys Gly Ser
545 550 555

Cys Gly Asp Leu Pro Gly Leu Arg Thr Ala Val Pro Pro His Glu
560 565 570

Pro Gly Gly Pro Gly Ser Pro Gly Gly Leu Gly Gly Gly Pro Ser
575 580 585

Ala Trp Glu Ala Cys Pro Pro Ala Leu Arg Gly Leu His His Asp
590 595 600

Leu Leu Leu Arg Lys Met Ser Ser Ser Ser Pro Asp Leu Leu Ser
605 610 615

Ala Ala Leu Gly Ser Arg Gly Arg Gly Ala Thr Gly Gly Ala Gly
620 625 630

Asp Pro Gly Ser Pro Pro Pro Ala Arg Gly Asp Thr Pro Pro Ser
635 640 645

Glu Gly Ser Ala Pro Gly Ser Thr Ser Pro Asp Ser Pro Gly Gly
650 655 660

Ala Lys Gly Glu Pro Pro Pro Pro Val Gly Pro Gly Glu Gly Val
665 670 675

Gly Leu Leu Gly Thr Gly Arg Glu Gly Thr Ser Gly Arg Gly Gly
680 685 690

Ser Arg Ala Gly Ser Gln His Leu Thr Pro Ala Ala Leu Leu Tyr
695 700 705

Arg Ala Ala Val Thr Arg Ser Gln Lys Arg Gly Ile Ser Ser Glu
710 715 720

Glu Glu Glu Gly Glu Val Asp Ser Glu Val Glu Leu Thr Ser Ser
725 730 735

Gln Arg Trp Pro Gln Ser Leu Asn Met Arg Gln Ser Leu Ser Thr
740 745 750

Phe Ser Ser Glu Asn Pro Ser Asp Gly Glu Glu Gly Thr Ala Ser
755 760 765

Glu Pro Ser Pro Ser Gly Thr Pro Glu Val Gly Ser Thr Asn Thr
770 775 780

Asp Glu Arg Pro Asp Glu Arg Ser Asp Asp Met Cys Ser Gln Gly
785 790 795

Ser Glu Ile Pro Leu Asp Pro Pro Pro Ser Glu Val Ile Pro Gly
800 805 810

Pro Glu Pro Ser Ser Leu Pro Ile Pro His Gln Glu Leu Leu Arg
815 820 825

Glu Arg Gly Pro Pro Asn Ser Glu Asp Ser Asp Cys Asp Ser Thr
830 835 840

Glu Leu Asp Asn Ser Asn Ser Val Asp Ala Leu Arg Pro Pro Ala
845 850 855

Ser Leu Pro Pro

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCATGAAAGC CACTCGTATT CCT 23

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCTTTCTGTT GATTATGTGG CGC

What is claimed is:

1. cDNA coding for mRNA which encodes a leucine-zipper protein kinase.

2. cDNA having a sequence at least about 50% homologous to the nucleic acid sequence set forth in SEQ ID NO: 1.

3. A protein produced from the cDNA of claim 2.

4. A substantially purified, isolated mammalian leucine-zipper protein kinase comprising a protein having a sequence at least about 50% homologous to the amino acid sequence set forth in SEQ ID NO: 2.

5. A pharmaceutical composition comprising leucine-zipper protein kinase in a pharmaceutically acceptable carrier.

6. A polynucleotide probe comprising a nucleic acid sequence substantially homologous to at least a portion of the cDNA of claim 2.

7. A polynucleotide probe comprising a nucleic acid sequence specifically hybridizable to at least a portion of the cDNA of claim 2.

8. A construct comprising a vector and at least a portion of the cDNA of claim 2.

9. The construct of claim 8 further comprising a promoter operably linked to said cDNA.

10. Recombinant host cells transformed with cDNA of claim 2 or a portion thereof whereby said host cells express a leucine-zipper protein kinase.

11. A method of producing leucine-zipper protein kinase which comprises culturing recombinant host cells wherein said host cells are transformed with cDNA having SEQ ID NO: 2 operably linked to regulatory control sequences which effect the expression of said coding sequence in said transformed host cells and isolating said leucine-zipper protein kinase produced by said host cells.

12. A method of detecting a gene coding for leucine-zipper protein kinase in a sample comprising contacting the sample with a probe of claim 6 or claim 7 under conditions which allow for the formation of a polynucleotide duplex between the probe and said gene, and detecting the presence or absence of a polynucleotide duplex whereby the presence of a polynucleotide duplex indicates the presence of said leucine-zipper protein kinase in said sample.

13. A method of inhibiting the hyperproliferation of neuronal cells comprising contacting said cells with an oligonucleotide substantially complementary to the nucleic acid sequence set forth in SEQ ID NO: 1.

14. A method of inhibiting the formation of protein-protein kinase complexes in a cell comprising contacting said cell with polypeptides having a sequence substantially homologous to at least a portion of the sequence set forth in SEQ ID NO: 2.

15. The method of claim 14 wherein said portion of the sequence set forth in SEQ ID NO: 2 comprises at least amino acids 442 through 468.

16. A method of inhibiting the formation of nucleic acid-protein kinase complexes in a cell comprising contacting said cell with polypeptides having a sequence substantially homologous to at least a portion of the sequence set forth in SEQ ID NO: 2.

17. The method of claim 16 wherein said portion of the sequence set forth in SEQ ID NO: 2 comprises at least amino acids 442 through 468.

18. A method of inhibiting the formation of protein-protein kinase complexes in a cell comprising contacting said cell with an oligonucleotide substantially complementary to a portion of the nucleic acid sequence set forth in SEQ ID NO: 1.

19. A method of inhibiting the formation of nucleic acid-protein kinase complexes in a cell comprising contacting said cell with an oligonucleotide substantially complementary to a portion of the nucleic acid sequence set forth in SEQ ID NO: 1.

20. A method of treating a mammal suffering from tumors of the central nervous system comprising administering to said mammal a therapeutically effective amount of an oligonucleotide having a sequence substantially complementary to a portion of the sequence set forth in SEQ ID NO: 1.

21. A method of treating a mammal suffering from tumors of the central nervous system comprising administering to said mammal a therapeutically effective amount of a polypeptide having a sequence substantially homologous to at least a portion of the sequence set forth in SEQ ID NO: 2.

22. The method of claim 21 wherein said portion of the sequence set forth in SEQ ID NO: 2 comprises at least amino acids 442 through 468.

1/3

1 [illegible]
74 [illegible]
M A C L H E T R T P S P S F G G 16
147 [illegible]
F V S T L S E A S M R K L D P D T S D C T P E K 40
220 [illegible]
D L T P T H V L Q L H E Q D A G G P G G A A G S P 65
293 [illegible]
E S R A S R V R A D E V R L Q C Q S G S G F L E 89
366 [illegible]
G L F G C L R P V W T M I G K A Y S T E H K Q Q 113
439 [illegible]
Q E D L W E V P F E E I L D L Q W V G S G A Q G A 138
512 [illegible]
V F L G R F H G E E V A V K K V R D L K E T D I 162
585 [illegible]
K H L R K L K H P N I I T F K G V C T Q A P C Y 186
658 [illegible]
C I L M E F C A Q G Q L Y E V L R A G R P V T P S 211
731 [illegible]
L L V D W S M G I A G G M N Y L H L H K I I $\boxed{H\ R}$ 235
804 [illegible]
$\boxed{D\ L}$ K S P $\boxed{N}$ M L I T Y D D V V $\boxed{K}$ I S $\boxed{D\ F\ G}$ T S K 259
877 [illegible]
E L S D K S T K M S F A G T V A W M $\boxed{A\ P\ E}$ V I R N 284
950 [illegible]
E P V S E K V $\boxed{D}$ I $\boxed{W\ S}$ F $\boxed{G}$ V V L W E L L T G E I 308
1023 [illegible]
P Y K D V D S S A I I W G V G S N S L H L P V P 332
1096 [illegible]
S S C P D G F K I L L R Q C W N S K P R N R P S F 357
1169 [illegible]
R Q I L L H L D I A S A D V L S T P Q E T Y F K 381
1242 [illegible]
S Q A E W R E E V K L H F E K I K S E G T C L H 405
1315 [illegible]

FIGURE 1 a

R L E E E L V M R R R E E L R H A L D I R E H Y E 430

1388 AAGGAGCTGGAGAGAGCCAACAACCTGTATATGGAACTTAATGCCCTCATGTTGCAGCTGGAACTCAAGGAG

R K L E R A N N L Y M E L N A L M L Q L E L K E 454

1461 AGGGAGCTGCTCAGGCGAGAGCAAGCTTTAGAGCGGAGGTGCCCAGGCCTGCTGAAGCCACACCCTTCCCGGG

R E L L R R E Q A L E R R C P G L L K P H P S R 478

1534 GCCTCCTGCATGGAAACACAATGGAGAAGCTTATCAAGAAGAGGAATGTGCCACAGAATCTGTCACCCCATAG

G L L H G N T M E K L I K K R N V P Q N L S P H S 503

1607 CCAAAGGCCAGATATCCTCAAGGCGGAGTCTTTGCTCCCTAAACTAGATGCAGCCCTGAGTGGGGTGGGGCTT

Q R P D I L K A E S L L P K L D A A L S G V G L 527

1680 CCTGGGTGTCCTAAGGCCCCCCCCTCACCAGGACGGAGTCGGCGTGGCAAGACCCGTCACCGCAAGGCCAGCG

P G C P K A P P S P G R S R R G K T R H R K A S 551

1753 CCAAGGGGAGCTGTGGGGACCTGCCTGGGCTTCGTACAGCTGTGCCACCCCATGAACCTGGAGGACCAGGAAG

A K G S C G D L P G L R T A V P P H E P G G P G S 576

1826 CCCAGGGGGCCTAGGAGGGGGACCCTCAGCCTGGGAGGCCTGCCCTCCCGCCCTCCGGGGCCTTCATCATGAC

P G G L G G G P S A W E A C P P A L R G L H H D 600

1899 CTCCTGCTCCGCAAAATGTCTTCATCGTCCCCAGACCTGCTGTCAGCAGCACTAGGGTCCCGGGGCCGGGGGG

L L L R K M S S S S P D L L S A A L G S R G R G 624

1972 CCACAGGGGGAGCTGGGGATCCTGGCTCACCACCTCCCGCCCGGGGTGACACCCCACCAAGTGAGGGCTCAGC

A T G G A G D P G S P P P A R G D T P P S E G S A 649

2045 CCCTGGCTCCACCAGCCCAGATTCACCTGGGGGAGCCAAAGGGGAACCACCTCCTCCAGTAGGGCCTGGTGAA

P G S T S P D S P G G A K G E P P P P V G P G E 673

2118 GGTGTGGGGCTTCTGGGAACTGGAAGGGAAGGGACCTCAGGCCGGGGAGGAAGCCGGGCTGGGTCCCAGCACT

G V G L L G T G R E G T S G R G G S R A G S Q H 697

2191 TGACCCCAGCTGCACTGCTGTACAGGGCTGCGGTCACCCGGAGTCAGAAACGTGGCATCTCATCGGAAGAGGA

L T P A A L L Y R A A V T R S Q K R G I S S E E E 722

2264 GGAAGGAGAGGTAGACAGTGAAGTAGAGCTGACATCAAGCCAGAGGTGGCCTCAGAGCCTGAACATGCGCCAG

E G E V D S E V E L T S S Q R W P Q S L N M R Q 746

2337 TCACTATCTACCTTCAGCTCAGAGAATCCATCAGATGGGGAGGAAGGCACAGCTAGTGAACCTTCCCCCAGTG

S L S T F S S E N P S D G E E G T A S E P S P S 770

2410 GCACACCTGAAGTTGGCAGCACCAACACTGATGAGCGCCCAGATGAGCGGTCTGATGACATGTGCTCCCAGGG

G T P E V G S T N T D E R P D E R S D D M C S Q G 795

2483 CTCAGAAATCCCACTGGACCCACCTCCTTCAGAGGTCATCCCTGGCCCTGAACCCAGCTCCCTGCCCATTCCA

S E I P L D P P P S E V I P G P E P S S L P I P 819

2556 CACCAGGAACTTCTCAGAGAGCGGGGCCCTCCCAATTCTGAGGACTCAGACTGTGACAGCACTGAATTGGACA

H Q E L L R E R G P P N S E D S D C D S T E L D 843

2629 ACTCCAACAGCGTTGATGCCCTTCGGCCCCCAGCTTCCCTCCCTCCATGAaagccactcgtattccttgtaca

N S N S V D A L R P P A S L P P * 859

2702 tagagaaatatttatatggattatatatatatacatatatatatatatatgcgccacataatcaacagaaaga

2775 tggggctgtcccagccgtaagtcaggctcgagggagactgatcccctgaccaattcacctgataaactctagg

2848 gacactggcagctgtggaaatgaatgaggcacagccgtagagctgtggctaagggcaagcccttcctgcccc

2921 accccattccttatattcagcaagcaacaaggcaatagaaaagccagggttgtctttatattctttatcccca

2994 aataataggggggtgggggggaggggcggtgggaggggcaggagagaaaaccacttagactgcacttttctgttc

3067 cgtttactctgtttacacatttgcacttgggaggagggaggctaaggctgggtcctcccctctgaggtttct

3140 caggtggcaatgtaactcatttttttgtcccaccatttatcttctctgcccaagccctgtcttaaggcccagg

3213 ggggaggttaggagactgatagcatgtgatggctcaggctgaagaaccggggttctgtttaagtccctgctttt

3286 atcctggtgcctgattggggtggggactgtcctactgtaacccctgtgaaaaaccttgaaaaataacactcca

3359 tgcaggaaaaaaaaaaaaaaaaaaaaaaa

FIGURE 1 b

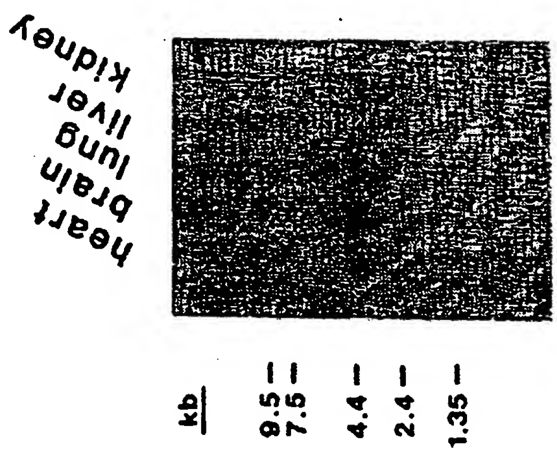

FIG. 2A(b)

FIG. 2B(b)

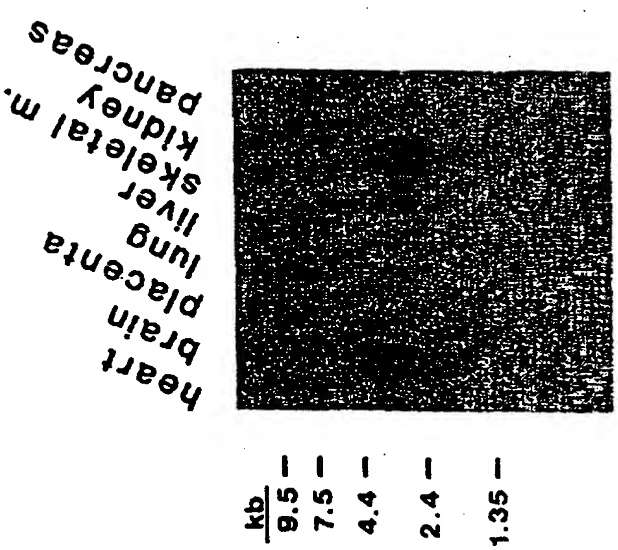

FIG. 2A(a)

FIG. 2B(a)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02792

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 9/12, 15/54
US CL :435/194; 536/23.2; 424/93.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/194; 536/23.2; 424/93.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, APS
search terms: leucine-zipper protein kinase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
NONE	NONE	NONE

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 MAY 1995

Date of mailing of the international search report

07 JUN 1995

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Form PCT/ISA/210 (second sheet)(July 1992)*

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-2, 8-11, 13 and 18-20, drawn to cDNA and first method of use.

Group II, claims 3-5, drawn to a leucine-zipper protein kinase.

Group III, claims 6-7 and 12, drawn to DNA probes and methods of use.

Group IV, claims 14-17 and 21-22, drawn to a method of inhibiting cells using the protein.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

Groups I and II are physically and chemically unrelated compounds, each with separate function and industrial applicability.

Group III is unrelated to the protein of Group II both chemically and physically, also with separate functions. The cDNA of Group I and the probes of Group III are separate, in that they are not necessarily physically the same, and each possess distinct function and industrial applicability from the other. Thus, they lack a corresponding special technical feature.

The method of Group IV does not employ the compounds of Groups I and III, and thus lacks a common special technical feature. The method of Group IV employs the protein of Group II, but the protein has separate function outside this particular method, and the method can be performed with another compound. Since the method does not necessarily require the specific protein, they lack a corresponding special technical feature.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.